April 17, 2002

Ms. Jane Scott-Smith
Office of Science Policy and Coordination
Office of Pollution Prevention and Toxic Substances
USEPA Headquarters, Mail Code 7201M
Ariel Rios Building
1200 Pennsylvania Avenue, N. W.
Washington, DC 20460

Re: Docket Number: Docket Control Number OPPTS-42212F
Issues Pertaining to Selection of Substances for Validation Studies of Screening Assays for EPA's Endocrine Disruptor Screening Program (EDSP)

Dear Ms. Scott-Smith:

The American Chemistry Council (ACC or the "Council") has played an active role in the development and implementation of the endocrine disruptor screening and testing program (EDSP) for several years[1]. The Council strongly supports the Agency's efforts to seek technical advice and recommendations from the Endocrine Disruptor Methods Validation Subcommittee (EDMVS) and the public concerning matters related to the validation of endocrine disruptor screening and testing methods. ACC encourages the timely development and implementation of a scientifically robust EDSP.

At the March meeting of the EDMVS, EPA specifically requested suggestions and comments on the selection of substances for standardization and validation studies of EDSP screening assays. The Council urges EPA to carefully consider the following comments and recommendations.

Sincerely,

Original Signed By

Richard A. Becker, Ph.D., DABT
Senior Director

[1] The Council represents more than 90 percent of the productive capacity for basic industrial chemicals within the United States and its members are the leading companies engaged in the business of chemistry. EPA's endocrine disruptor screening and testing program (EDSP) may significantly affect the Council and its members. For that reason, the Council and its members have attempted to assist the Agency in developing and implementing its EDSP. In that regard, ACC and its members actively participated in EDSTAC and are actively participating in the EDMVS.

The American Chemistry Council believes the Agency's first step towards evaluating substances to be used in standardizing and validating specific Tier 1 screening methods for the EPA's EDSP should be to develop criteria to select substances for the standardization and validation studies. At this stage of early protocol development, the emphasis should be on using relatively well-characterized substances. Such substances should allow the Agency, FACA, and others to assess two essential aspects of the data to be generated: 1) the early performance and long-range promise of a particular protocol and 2) the commonality or differences of the protocols. ACC recommends the following selection criteria for consideration by the Agency. (Note – these criteria are for Tier 1 assay standardization & validation studies. Evaluation of Tier 2 tests may need dramatically different criteria and substances.**)**

1. The hormonal activity and mechanism of hormonal effect of a substance should already be known from both *in vitro* and *in vivo* research methods. There must be sufficient and robust information and data from scientific reports on each substance with respect to the hormonal mode of action, the hormonal potency and specificity and ADME[2] characteristics. These data enable a prediction of results for the screening method and a reasonable assessment of protocol performance.

2. Substances selected must be readily available through commercial vendors. These substances are likely to be used over a number of years, in several protocols and by a number of laboratories s part of the standardization and validation program. Further, other labs will have an interest to establish and demonstrate their proficiency with these screening methods. Therefore, it is necessary to select substances which will be readily available through commercial sources presently and in the future.

3. The Agency must focus on substances with known estrogen, androgen and thyroid (EAT) activity, consistent with the Agency's EDSP Statement of Policy. The priority for the EDSP should be estrogen, androgen and thyroid hormonal activities or modes of action. The focus should be on direct modes of EAT actions and should include receptor agonists/antagonists and, if applicable, hormone synthesis inhibitors. Importantly, the Agency should avoid use of substances that exert endocrine effects via indirect modes or mechanisms (except to establish specificity, as described in point 7 below).

[2] Absorption, distribution, metabolism and excretion

4. Substances with high specificity (either as agonists or antagonists) are preferred and should be used to the maximum extent practicable. In cases where the use of a mixed agonist/antagonist is necessary or where there are other overlapping specificities, EPA must select the concentrations and doses carefully, keeping in mind the effects such mixed activities may have upon the type, magnitude and nature of the response(s).

5. Substances with particular EAT activity should be evaluated in the appropriate screening method. While there may be some overlap, it is not necessary to use exactly the same set of substances in the validation of each screening method. For example, substances with estrogenic activity should be used for validation of the uterotrophic assay, but it would make no sense to use the same complete set of substances in the Hershberger assay for androgens.

6. In general, validation must cover the entire range of activities anticipated from the population of substances that will be selected to be evaluated with the assay. Little or no confidence can be placed upon results of substances whose activities fall outside the activities or modes of action of the set of substances for which the assay has been validated. Further, the set of substances used for development and standardization of an assay should be different from the set of substances used for validation. In the validation series, the substances selected should include materials with a range of potencies; from strong to weak to completely negative for the appropriate EAT mechanisms.

7. It is essential to address the issue of specificity (false positive responses) in the validation studies of each assay. In particular, since the EDSP screening assays and the Tier 1 battery have been selected by EPA to minimize or eliminate false negatives, such characteristics will likely generate false positives. Therefore, in the validation of EDSP screening assays, it is critical to include substances that exert effects (and/or toxicity) by mechanisms that are not primarily hormonal in order to establish the specificity of the assay endpoints (e.g., evaluate potential for false positive responses due to a non-hormonal toxicity). In some cases it may be beneficial to establishing specificity by evaluating, for example, a pure estrogen agonist in an assay designed for androgens (and vice versa).

8. EPA must coordinate its activities with the OECD EDTA with respect to study design, selection of substances and dose levels for assay validation. OECD has initiated (and for some assays, largely completed) validation studies using specific chemical substances. EPA's activities with respect to assay validation for the EDSP should demonstrate the Agency's strong support of international harmonization and mutual acceptance of data.

9. The approach EPA adopts for standardization and validation should be sufficiently rigorous to comply with generally recognized scientific principles of study design and conduct. With respect to test articles selected for EDSP validation, this should include knowledge of chemical purity, stability and concentration (particularly the applied or administered dose). In evaluating substances for potential selection for use in particular assays and routes of administration, EPA should consider what degree of analytical chemistry would be necessary to meet these recognized scientific standards.

10. In compiling substances for standardization and validation, EPA must appropriately qualify and characterize any and all such lists. EDSTAC spent a great deal of time and effort addressing communications issues, and EPA should implement the EDSTAC recommendations to ensure proper understanding by the public of such a list of substances. The Table must be qualified and include a disclaimer along the lines of: ***"****Inclusion of a substance in this table does not mean that EPA has or will make a determination that any of the uses of the chemical will pose a significant risk. Further, this table should not be taken as a list of "endocrine disruptors." The substances listed are simply compounds which have, or may prove to be, useful in developing, standardizing or validating screening and testing methods."*

11. Each entry in which reference is made to a particular hormonal mechanism of action or to potency or activity must be referenced. This is necessary for transparency and accuracy. This would permit members of the EDMVS (and the public) to readily access the citation and to review the actual study results (study design, dose levels, endpoints measured and results). This is critical and is necessary for selection of chemicals and dose levels for prevalidation studies – it also important for constructing the predictive models.